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Nifedipine-morphine interaction: a further investigation on nociception and locomotor activity in mice

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Abstract—Nociception and locomotor activity were tested in mice (C57BL/6 and DBA/2 strains), receiving the dihydropyridine calcium-channel blocker nifedipine, alone or combined with morphine. The calcium antagonist did not change the reaction time to thermal stimulation (tail-flick test), when administered alone, but combinations of nifedipine and morphine prolonged tail-flick latencies less than did the opiate alone. Nifedipine decreased locomotion in both strains, reduced the hypermotility induced by morphine in C57 mice, and enhanced the locomotor depression induced by the opiate in DBA mice. A comparison of the effects of nifedipine with those of the non-calcium antagonist vasodilator, hydralazine, suggests that the interactions with morphine were not exclusively related to neuronal changes produced by calcium channel blockade, but also to haemodynamic factors. In fact, except for the lack of interference with morphine-induced hypermotility in C57 mice, hydralazine, given alone or in combination with morphine, produced effects similar to those of nifedipine.

Calcium ions are involved in the effects of the opiates (Chapman & Way 1980) and calcium-channel blockers may interfere with these effects. In particular, it has been reported that calcium antagonists potentiate the antinociceptive action of opiates (Benedek & Szikszay 1984; Hoffmeister & Tettenborn 1986; Del Pozo et al 1987; Contreras et al 1988) and attenuate morphine-induced hypermotility (Martin et al 1990).

In the present study the analgesic and behavioural effects of morphine, given alone or in combination with nifedipine, a dihydropyridine calcium-channel blocker, were tested in mice belonging to the two inbred strains, C57BL/6 (C57) and DBA/2 (DBA). These two mouse strains were chosen for their different reactivity to opiates: DBA mice are more sensitive than C57 mice to the analgesic action of opiates; on the other hand, opiates produce hypermotility in C57 mice, but depress locomotor activity in DBA mice (Oliverio et al 1984). The effects of nifedipine were compared with those of hydralazine, a non-calcium antagonist vasodilator (Rudd & Blashke 1985), in order to verify whether the interaction with morphine was specifically related to calcium channel blockade.

Materials and methods

Animals. Naive male mice, 9–10 weeks old, of the inbred C57 and DBA strains (Charles River, Italy) were housed in standard transparent plastic cages (8 per cage), under standard animal room conditions (12 h light/dark cycle, ambient temperature of 23°C) with free access to food and water for at least one week before the experiment. The experiments were carried out between 0900 and 1400 h using different animals for different tests. Each experimental group included 8 mice.

Drugs. Nifedipine, dissolved in 50% polyethylene glycol (PEG), mol. wt 400, and hydralazine hydrochloride, dissolved in distilled water, were injected intraperitoneally in a volume of 4 mL kg^{-1}; control animals received 50% PEG. Morphine hydrochloride, dissolved in distilled water, and saline (0·9% NaCl) were injected intraperitoneally in a volume of 10 mL kg^{-1}.

Nociception. Nociception was assessed using a radial heat tail-flick test. The mean of the tail-flick latencies (TFL), measured in three predrug trials, represented the individual baseline. Only animals showing TFL ranging from 3 to 5 s were included in the experiment. Immediately after baseline assessment, mice received a first injection with PEG, nifedipine (2·5 or 5 mg kg^{-1}) or hydralazine hydrochloride (1 mg kg^{-1}) and, 15 min later, a second injection, which contained morphine hydrochloride (5 mg kg^{-1}) or saline. Postdrug TFL was measured 15, 30, 45 and 60 min after morphine (or saline) and were expressed as a percentage of the maximum possible effect, according to the formula

$$\% \text{ maximum possible effect} = [(\text{TFL-baseline})/(10 - \text{baseline}) \times 100],$$

in which "10" represented the cut-off time in s. The results were statistically evaluated by a two-way analysis of variance for each strain, the factors being treatment (8 levels) and time from morphine administration (repeated measures: 4 levels). Individual between-treatment comparisons were carried out with the Duncan multiple-range test.

Locomotor activity. The locomotor activity was measured in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20 × 10 cm compartments connected by a 3 × 3 cm opening. For each mouse the number of crossings from one compartment to the other was automatically recorded by means of a microswitch connected to the tilting floor of the box. The apparatus was located in a sound-insulated cubicle.

C57 mice received a first injection with PEG, nifedipine (2·5, 5 or 10 mg kg^{-1}) or hydralazine hydrochloride (1 mg kg^{-1}) and a second injection with saline or morphine hydrochloride (10 mg kg^{-1}), 15 min later. The animals were subjected to a 30 min activity test, 15 min after the second injection. In DBA mice the highest dose of nifedipine (10 mg kg^{-1}) was excluded, while morphine was tested also at a lower dose (5 mg kg^{-1}), because of the strong depressant effects produced by combinations of the two drugs.

Median number of crossings and semi-interquartile range were calculated for each experimental group. Significance of the differences between groups was evaluated by means of the Mann-Whitney U-test.

Results

Nociception. Fig. 1 shows tail-flick latencies, measured at various time intervals after morphine administration, in mice pretreated with nifedipine or hydralazine.

C57 strain. A two-way analysis of variance gave significant treatment ($F(7,168):22.88$, $P<0·001$) and testing time ($F(3,168):4.05$, $P<0·01$) main effects and a significant treatment × time interaction ($F(21,168):1.67$, $P<0·05$). A further analysis with Duncan's test showed that nifedipine and hydralazine alone had no effect, while morphine prolonged tail-flick latencies during the whole testing time. When morphine was given after hydralazine or after the highest dose of nifedipine (5 mg kg^{-1}), tail-flick latencies were shorter than those observed in mice receiving the opiate alone (Fig. 1).

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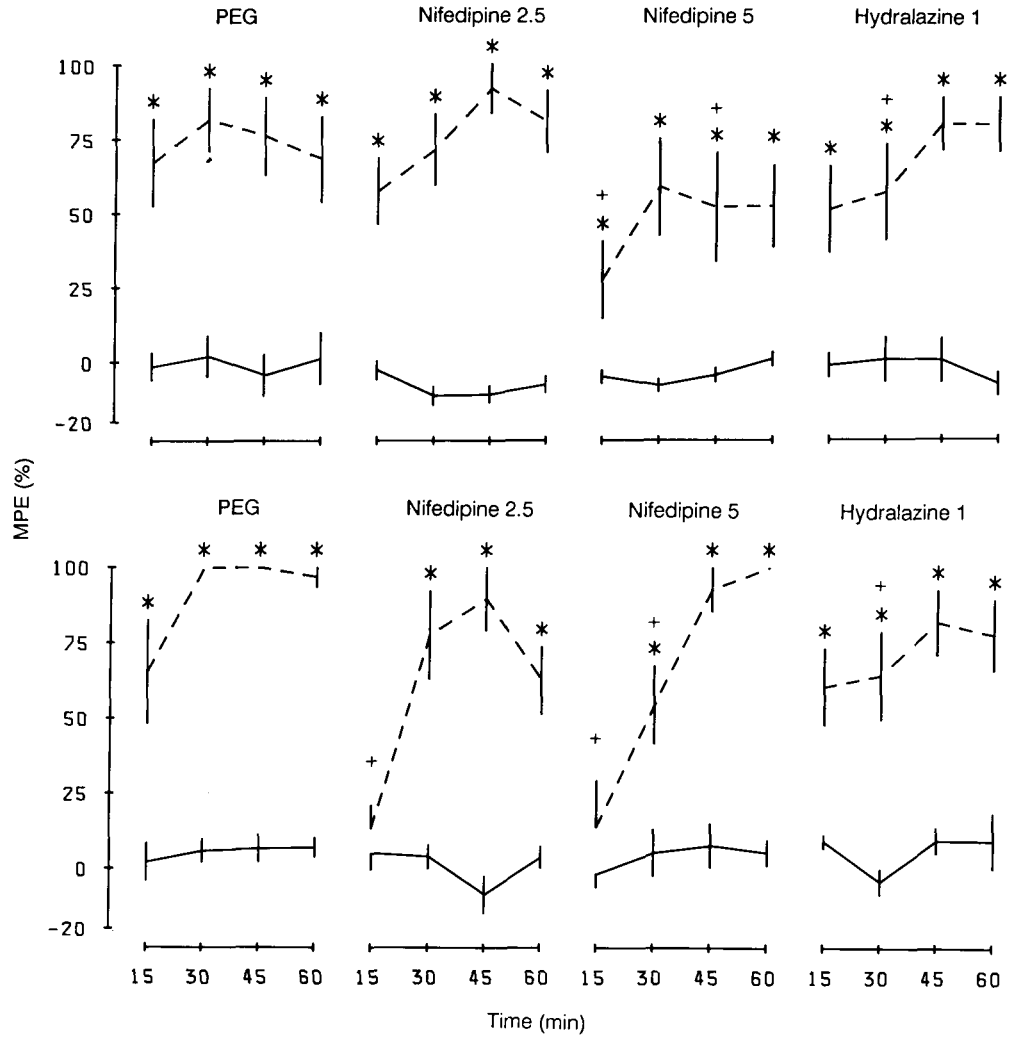

FIG. 1. Tail-flick latencies in mice of C57BL/6 and DBA/2 strains, receiving saline solutions (—) or morphine hydrochloride (---) (5 mg kg^{-1}) 15 min after PEG, nifedipine (2·5 or 5 mg kg^{-1}) or hydralazine hydrochloride (1 mg kg^{-1}). Postdrug tail-flick latencies, expressed as a percentage of maximum possible effect (% MPE), were measured 15, 30, 45 and 60 min after the second injection. Values are the means ± s.e. of 8 mice. $*P < 0.05$ morphine compared with saline; $+P < 0.05$ nifedipine and hydralazine compared with PEG.

DBA strain. A two-way analysis of variance showed treatment ($F(7,168):39.28$, $P < 0.001$) and testing time ($F(3,168):26.67$, $P < 0.001$) main effects and a significant treatment × time interaction ($F(21,168):6.22$, $P < 0.001$). As in C57 mice, nifedipine and hydralazine alone had no effect, while morphine induced a stronger antinociceptive action, reaching the maximum 30 min after administration. Prolongation of tail-flick latencies was less evident, or even disappeared (first postdrug measure), in mice receiving morphine after nifedipine or hydralazine (Fig. 1).

Locomotor activity. C57 strain. Fig. 2 shows that nifedipine (2·5, 5 and 10 mg kg^{-1}) and hydralazine (1 mg kg^{-1}), given alone (saline as second injection), significantly reduced the number of activity crossings. Morphine (10 mg kg^{-1}) strongly stimulated locomotor activity in PEG pretreated mice. Morphine-induced hypermotility was significantly attenuated, but not abolished, by all doses of nifedipine, but was not affected by hydralazine.

DBA strain. Locomotor activity was depressed by nifedipine (2·5 and 5 mg kg^{-1}) and hydralazine (1 mg kg^{-1}), and by morphine (5 and 10 mg kg^{-1}). Combinations of nifedipine, or hydralazine, with the highest dose of morphine, produced stronger depressant effects and almost abolished locomotion (Fig. 2).

Discussion

In the present study, the dihydropyridine calcium-channel blocker nifedipine had no analgesic effect when given alone, but prevented the prolonging action of morphine on tail-flick latencies in C57 mice and, to a greater extent, the DBA strain. In the locomotor activity test, nifedipine decreased activity in both strains, reduced the hypermotility induced by morphine in the C57 strain and enhanced the locomotor depression produced by the opiate in DBA mice.

Failure of nifedipine to affect mouse responses in the radial heat tail-flick test is in agreement with previous findings indicating that calcium-channel blockers produce antinociception in the acetic acid-induced abdominal constriction test (Del Pozo et al 1987; Ohnishi et al 1988), but not in the hot-plate test,

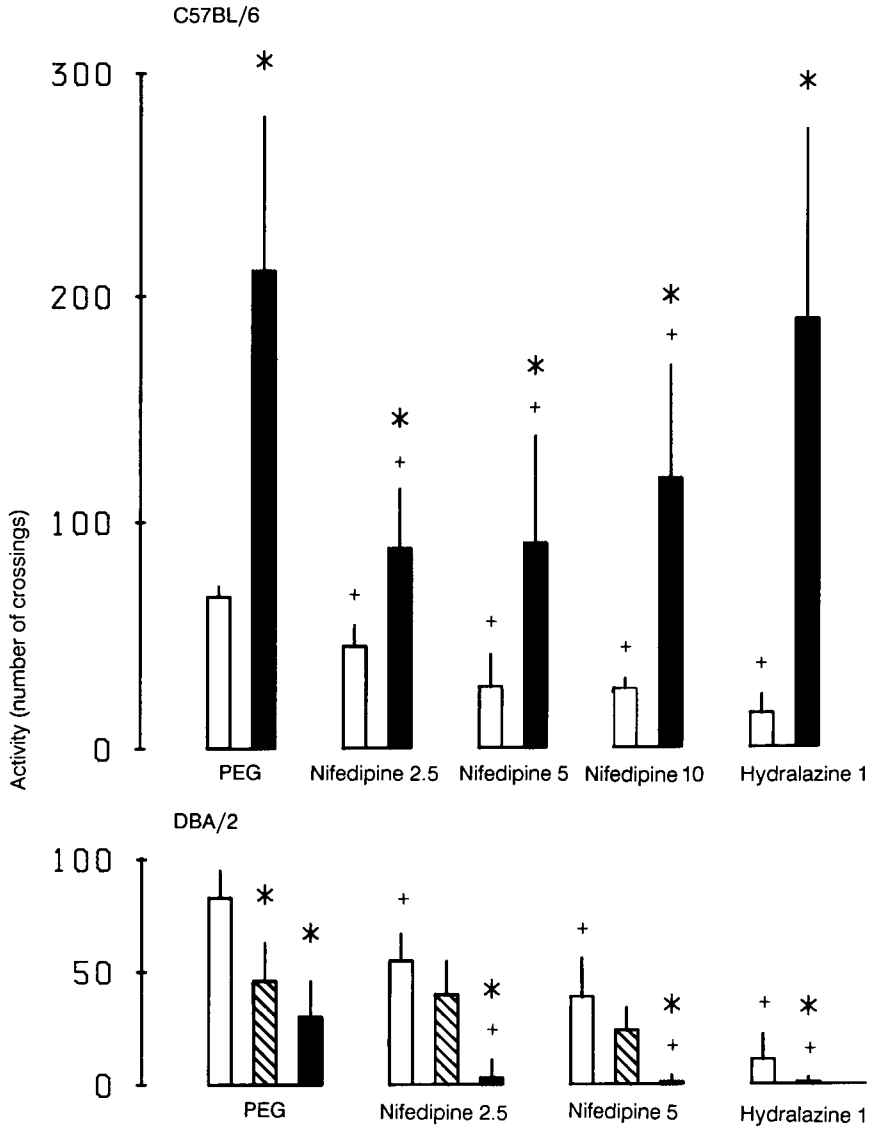

FIG. 2. Locomotor activity measured, during 30 min, in mice of C57BL/6 and DBA/2 strains, receiving saline □ or morphine hydrochloride (▩ 5, or ■ 10 mg kg^{-1}) 15 min after PEG, nifedipine (2·5, 5 or 10 mg kg^{-1}) or hydralazine hydrochloride (1 mg kg^{-1}). The activity test started 15 min after the second injection. Columns represent median crossings in groups of 8 mice. Vertical lines indicate semi-interquartile ranges. $*P < 0{\cdot}05$ morphine compared with saline; $+P < 0{\cdot}05$ nifedipine and hydralazine compared with PEG.

another test based on thermal stimulation (Benedek & Szikszay 1984; Hoffmeister & Tettenborn 1986; Contreras et al 1988). Conversely, prevention of morphine action on tail-flick responses by nifedipine contrasts with previous findings, showing potentiation of the antinociceptive effects of morphine and other opiates by calcium antagonists (Benedek & Szikszay 1984; Hoffmeister & Tettenborn 1986; Del Pozo et al 1987; Contreras et al 1988). However, Contreras et al (1988) observed that the analgesic response to morphine, in mice subjected to the hot plate test, was increased by various calcium-channel blockers, with the exception of nifedipine, which exerted an antagonistic action. Those authors supposed that nifedipine could interfere with the analgesic effects of morphine independently from its calcium-channel blocking activity, as suggested for other pharmacological actions of the drug (Swanson & Green 1986; Colado et al 1991). The present findings suggest that haemodynamic factors may be involved in the nifedipine–morphine interaction, since nifedipine and hydralazine exerted similar effects, when combined with the opiate, in the tail-flick test. Nifedipine and hydralazine, when combined with morphine, might produce similar haemodynamic changes or might modify the pharmacokinetics of the opiate and its concentration in spinal and supraspinal sites of the antinociceptive action. In this respect, it must be considered that production of analgesia by systemically administered morphine is a result of a multiplicative spinal–supraspinal interaction (Yeung & Rudy 1980). It also seems important to note that spinal administration of the calcium antagonist verapamil inhibited the antinociceptive action of systemic morphine in the tail-flick test (Lux et al 1988).

Haemodynamic factors might also be involved in the locomotor effects of nifedipine and morphine combined, in DBA more than in C57 mice. In agreement with previous findings (Czyrak et al 1990; Martin et al 1990), nifedipine reduced locomotor activity in both mouse strains. In C57 mice, morphine

induced a locomotor hyperactivity that was reduced by nifedipine. The locomotor depressant action of nifedipine could have contributed to the reduction of morphine-induced hypermotility, but it must be noted that a depressant dose of hydralazine did not affect the stimulant action of the opiate. Thus, the partial antagonistic action, exerted by nifedipine on morphine-induced hypermotility, might be, at least in part, due to calcium channel blockade, as suggested by previous findings indicating that calcium antagonists specifically interfere with the locomotor stimulant effects of opioids (Martin et al 1990). Contrary to what happened in C57 mice, in DBA mice, both nifedipine and morphine reduced locomotor activity, with stronger depressant effects after drug combination. Hydralazine, given alone or in combination with morphine, produced locomotor depressant effects similar to those exerted by nifedipine. In both cases, the mice did not exhibit any stereotyped behaviour that might explain the inhibitory action of the drugs on locomotor activity. DBA mice receiving combinations of the highest doses of nifedipine (or hydralazine) and morphine appeared deeply depressed till the end of the activity test and were almost motionless even when left in the activity cages for a further 30 min. The opposite locomotor effects, produced by morphine in C57 and DBA mice, have been ascribed to neurochemical strain differences in response to the opiates (Oliverio et al 1984). However, since morphine can produce vasodilatation and hypotension (Jaffe & Martin 1985), it cannot be excluded that DBA mice are more sensitive than C57 mice to the hypotensive effects of the opiate. In this case, nifedipine (or hydralazine) and morphine might exert a synergistic reduction in blood pressure, which may account for the behavioural depression. It must be noted that nifedipine (duodenal application) significantly decreased blood pressure in normotensive rats, even at the dose of 5 mg kg^{-1} (Rao & Fonteles 1991), and that the hypotensive effects induced by verapamil, another calcium antagonist, were potentiated by a dose of morphine that, if given alone, produced only a minor reduction of arterial blood pressure (Della Puppa et al 1989). The hypothesis that a fall in blood pressure may play a role in the strong reduction of locomotor activity, induced by combinations of nifedipine and morphine in DBA mice, is supported by previous findings suggesting that circulatory changes may be involved in the behavioural interaction of the calcium antagonist with drugs affecting blood pressure, such as barbiturates at hypnotic doses (Sansone et al 1992). This does not exclude the possibility that some behavioural actions of calcium-channel blockers may be independent of their hypotensive effects (Bolger et al 1986; Turkkan & Hienz 1990).

In conclusion, the present findings confirm that calcium channel antagonists interfere with the effects of the opiates, but also suggest that such interference is not always a peculiarity of this kind of cardiovascular agent. In fact, the dihydropyridine calcium-channel blocker nifedipine and the non-calcium channel antagonist vasodilator hydralazine displayed a similar interaction with morphine in the tail-flick test, and enhanced similarly the locomotor depression induced by the opiate in DBA mice. Conversely, calcium channel blockade seems to play a more specific role in the attenuation by nifedipine of morphine-induced hypermotility in C57 mice, a behavioural stimulation not changed by hydralazine.

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